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(71) Applicant *(for all designated States except AT US)*: NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH–4058 Basel (CH).

(71) Applicant *(for AT only)*: NOVARTIS–ERFINDUNGEN VERWALTUNGS GESELLSCHAFT M.B.. [AT/AT]; Brunner Strasse 59, A–1230 Vienna (AT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BRAZZELL, Romulus, Kimbro [US/US]; 9705 Foxworth Drive, Alpharetta, GA 30201 (US). WOOD, Jeanette, Marjorie [NZ/CH]; In den Kleematten 18, CH–4105 Biel–Benken (CH). CAMPOCHIARO, Peter, Anthony [US/US]; 920 West Lake Avenue, Baltimore, MD 21210 (US). KANE, Frances, Elizabeth [US/US]; 3679 Maple Forge Ln., Gainesville, GA 30504 (US).

(74) Agent: BECKER, Konrad; Novartis AG, Corporate Intellectual Property, Patent & Trademark Department, CH–4002 Basel (CH).

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(54) Title: USE OF STAUROSPORINE DERIVATIVES FOR TREATING OCULAR NEOVASCULAR DISEASES

(57) Abstract

The invention provides a method for treating or preventing ocular neovascularization. The method administers an effective amount of a staurosporine derivative to treat or prevent retinal or choroidal neovascularization.

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USE OF STAUROSPORINE DERIVATIVES FOR TREATING OCULAR NEOVASCULAR DISEASES

The present invention relates in particular to the use of staurosporine derivatives in the preparation of a medicament for treating ocular neovascular disease, e.g., retinal neovascularization and choroidal neovascularization.

The retina of the eye receives its blood supply from two vascular beds, the retinal vessels which supply the inner two thirds of the retina, and the choroidal vessels which supply the outer one third. Damage to retinal blood vessels resulting in closure of retinal capillaries occurs in several disease processes including diabetic retinopathy, retinopathy of prematurity, branch retinal vein occlusion, and central retinal vein occlusion; they are collectively referred to as ischemic retinopathies. Retinal ischemia results in release of one or more angiogenic factors that stimulate neovascularization. The new vessels break through the internal limiting membrane (ILM) that lines the inner surface of the retina and grow along the outer surface of the vitreous. They recruit many other cells and produce sheets of vessels, cells, and extracellular matrix that exert traction on the retina, often leading to retinal detachment and severe loss of vision.

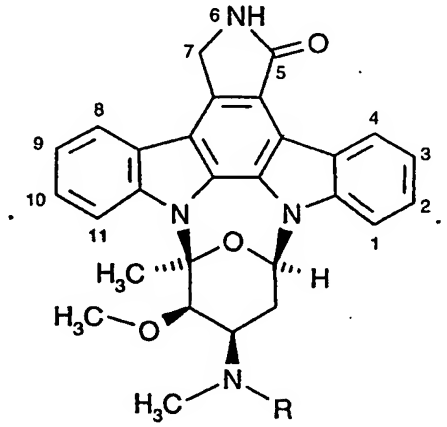
Choroidal neovascularization occurs in a number of disease processes, the most common of which is age-related macular degeneration. In this condition, the macula, which is especially adapted for acute and detailed vision, is damaged by gradual death of photoreceptor and RPE cells. This constitutes the degeneration part of the disease which results in the gradual loss of central vision. The reason for the cell death is unknown and there is currently no treatment. As the degeneration occurs, there is a tendency for new blood vessels to grow from the choroid to invade the sub-RPE and subretinal spaces. This process is called choroidal neovascularization (CNV) and it often leads to rapid and severe loss of vision from bleeding and scarring. If the CNV is well-delineated and not beneath the center of the fovea, which is true for a small minority of patients, laser treatment can sometimes help. Even when laser is initially successful, there is a high rate of recurrent CNV and loss of vision. A treatment directed at the stimuli for blood vessel growth is needed and would benefit patients with either retinal or choroidal neovascularization.

There is provided in accordance with the present invention a method for treating or preventing ocular neovascular diseases, including retinal neovascularization and choroidal neovascularization. The method has the step of administering an effective amount of a staurosporine derivative or a salt thereof.

The staurosporine treatment of the present invention is highly effective in inhibiting and preventing ocular neovascularization, unlike prior art laser treatment that has a limited efficacy. In addition, the staurosporine treatment is simple to administer, unlike prior art treatment methods, e.g., laser treatment, that are invasive and require complex equipment.

The present invention provides a method for treating ocular neovascular diseases. The method uses a medicament containing a staurosporine derivative. It has now surprisingly been found that the compounds of formula (I) are highly useful for treating ocular neovascularization, including retinal neovascularization and choroidal neovascularization.

Accordingly, the present invention relates to the use of a compound of formula (I),



(I),

wherein R represents a hydrocarbyl radical R^O or an acyl radical Ac,
in the preparation of a medicament for the treatment and/or prevention of ocular neovascular diseases.

Suitable hydrocarbyl radicals include acyclic, carbocyclic and carbocyclic-acyclic hydrocarbyl radicals having a maximum total number of carbon atoms of preferably 30, especially 18. Additionally suitable hydrocarbyl radicals are heterocyclic radicals and

heterocyclic-acyclic radicals. The hydrocarbyl radicals (R^o) may be saturated or unsaturated and substituted or unsubstituted. Suitable acyl radicals include optionally functionally modified carboxylic acid and organic sulfonic acid, and optionally esterified phosphoric acid, e.g., pyro- or ortho-phosphoric acid.

Preferred acyclic hydrocarbyl radicals include C_1-C_{20}-alkyl radicals; C_2-C_{20} hydroxyalkyl radicals of which the hydroxy group is in any position other than the 1-position; cyano-[C_1-C_{20}]-alkyl radicals; carboxy- [C_1-C_{20}]-alkyl radicals of which the carboxy group; and C_3-C_{20}-alkenyl radicals of which the free valency is not at the same carbon atom as the double bond. Exemplary acyclic hydrocarbyl radicals are radicals of lower alkyl, e.g., methyl, ethyl, propyl, butyl, pentyl, hexyl and heptyl; lower alkenyl, e.g., propenyl, 2- or 3-methallyl and 2- or 3-butenyl; lower alkadienyl, e.g., 1-penta-2,4-dinyl; and lower alkynyl, e.g., propargyl or 2-butynyl. Preferred carbocyclic hydrocarbyl radicals are radicals of mono-, bi- or polycyclic cycloalkyl, e.g., cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, bicyclo[2,2,2]octyl, 2-bycyclo[2,2,1]heptyl and adamantyl; cycloalkenyl; cycloalkandienyl; and corresponding aryl. Aryl radicals include radicals of phenyl, napthyl (e.g., 1- or 2-napthyl), biphenylyl (e.g., 4-biphenylyl), anthryl, fluorenyl, azulenyl, and aromatic analogues thereof having one or more saturated rings. Preferred carbocyclic-acyclic radicals are acyclic radicals that carry one or more of carbocyclic radicals. Heterocyclic radicals and heterocyclic-acyclic radicals include monocyclic, bicyclic, polycyclic, aza-, thia-, oxa-, thaza-, oxaza-, diaza-, triaza-, and tetraza-cyclic radicals of aromatic character.

Exemplary acyl radicals derived from an optionally functionally modified carboxylic acid (Ac^1) have the formula Z-C(=W)- in which W is oxygen, sulfur, or imino and Z is hydrogen, hydrocarbyl R^o, hydrocarbyloxy R^oO, or amino. Preferably, W is oxygen or sulfur, and Z is C_1-C_7 alkyl, especially C_1-C_4 alkyl, which is optionally substituted by halogen, carboxy or C_1-C_4 alkoxycarbonyl. Additionally preferred Z is phenyl, pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which is unsubstituted or substituted by C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4 alkoxycarbonyl, methylenedioxy, cyano and/or a salt thereof. Preferred Ac^1 acyl radicals have the formula R_b^o-CO-, in which R_b^o is hydrogen, benzoyl, or a hydrocarbyl radical, e.g., C_1-C_{19} alkyl radical which is optionally substituted by a carboxy group, cyano group, ester group, amino group or helogen. Another group of preferred Ac^1 acryl radicals

have the formula R^O-O-CO-. Yet another group of preferred Ac^1 acryl radicals have the formula

$$R_1 - N(R_2) - C(=W) -$$

in which R_1 and R_2 are independently selected from hydrogen and unsubstituted acyclic C_1-C_7 hydrocarbyl, preferably C_1-C_4 alkyl and C_3-C_7 alkenyl. R_1 and R_2, independently, can be monocyclic aryl, aralkyl or aralkenyl having a maximum of 10 carbon atoms, each of which is optionally substituted by C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen and/or nitro. Particularly desirable radicals of this group have hydrogen as R_1 and optionally substituted C_1-C_4 alkyl, C_3-C_7 alkenyl, phenyl, pyridyl, pyrimidyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl as R_2.

Exemplary acyl radicals derived from an organic sulfonic acid (Ac^2) have the formula R^O-SO_2- in which R^O is a hydrocarbyl radical. Preferably, R^O of the sulfonic acid acyl radicals is C_1-C_7 alkyl, phenyl, pyridyl, pyrimidyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which is unsubstituted or is substituted by C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4 alkoxycarbonyl, methylenedioxy, cyano and/or a salt thereof.

Exemplary acyl radicals derived from optionally esterified phosphoric acid (Ac^3) have the formula

$$R_1O - P(=O)(R_2O) -$$

in which R_1 and R_2 are independently selected from hydrogen, unsubstituted acyclic C_1-C_7 hydrocarbyl, preferably C_1-C_4 alkyl and C_3-C_7 alkenyl. R_1 and R_2, independently, can be monocyclic aryl, aralkyl or aralkenyl having a maximum of 10 carbon atoms, each of which is optionally substituted by C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen and/or nitro.

Additionally suitable staurosporine derivatives include staurosporines of formula (I) in which R is derived from an α-amino acid selected from glycine, phenylglycine, alanine, phenylalanine, proline, leucine, serine, valine, tyrosine, arginine, histidine and asparagine, or a salt thereof. Suitable staurosporine derivatives for the present invention are disclosed in further detail in U.S. Pat. No. 5,093,330 to Caravatti et al. The description of the

staurosporine derivatives in the patent is herein incorporated by reference.

Particularly preferred staurosporine derivatives of formula (I) for the present invention include:
N-(3-carboxypropionyl)-staurosporine, N-benzoyl-staurosporine, N-trifluoracetyl-staurosporine, N-methylaminothiocarbonyl-staurosporine, N-phenylcarbamoyl-staurosporine, N-(3-nitrobenzoyl)-staurosporine, N-(3-fluorobenzoyl)-staurosporine, N-tert-butoxycarbonyl-staurosporine, N-(4-carboxybenzoyl)-staurosporine, N-(3,5-dinitrobenzoyl)-staurosporine, N-alanyl-staurosporine, N-ethyl-staurosporine, N-carboxymethyl-staurosporine, N-[(tert.-butoxycarbonylamino)-acetyl]-staurosporine, N-(2-aminoacetyl)-staurosporine, and pharmaceutically acceptable salts thereof.

The pharmaceutical composition of the present invention which contains a compound of formula I as the active ingredient can be administered enterally, nasally, buccally, rectally, topically, orally, and parenterally, e.g., intravenous, intramuscular, intravitreal, sub-conjunctival or subcutaneous administration, to treat ocular neovascularization in mammalian subjects, especially human. The compositions may contain the active ingredient alone or, preferably, the active ingredient along with a pharmaceutically acceptable carrier. The effective dosage of the active ingredient depends on the type of targeted disease, as well as the species, age, weight and physical condition of the subject, pharmacokinetic data, and the mode of administration.

The compounds of formula I is administered in an amount effective against pathological conditions of a mammal, e.g., human. For an individual having a bodyweight of about 70 kg, the daily systemic dose administered is from about 0.1 g to about 20 g, preferably from about 0.5 g to about 5 g, of the active ingredient, and suitable pharmaceutical compositions may have from about 1% to about 95% of the active ingredient. Suitable unit dose forms include coated and uncoated tablets, ampoules, vials, suppositories, or capsules. Other suitable dosage forms include injectables, intraocular devices, intravitreal devices, ointments, creams, pastes, foams, tinctures, lip-sticks, eye-drops, oral-drops, sprays, dispersions and the like. The pharmaceutical compositions of the present invention are prepared in a manner known in the art, for example, by means of conventional mixing, granulating, coating, dissolving or lyophilizing processes.

Preference is given to the use of solutions of the active ingredient, and also suspensions or dispersions, especially isotonic aqueous solutions, dispersions or suspensions. Suitable pharmaceutical compositions containing the active ingredient may have carriers, e.g., mannitol and starch, preservatives, stabilizers, wetting agents, emulsifiers, solubilizers, salts for regulating osmotic pressure, buffers and the like. The compositions are prepared in a manner known in the art, for example by means of conventional dissolving and lyophilizing processes. A solution or suspension form of the composition may contain viscosity-increasing agents, e.g., sodium carboxymethylcellulose, carboxymethylcellulose, dextran, polyvinylpyrrolidone, and gelatins; and solubilizers, e.g., Tween 80 [polyoxyethylene(20)sorbitan mono-oleate; trademark of ICI Americas, Inc, USA].

Suitable carriers include fillers, e.g., sugars, for example lactose, saccharose, mannitol or sorbitol; cellulose preparations; calcium phosphates, e.g., tricalcium phosphate and calcium hydrogen phosphate; binders, e.g., starches, methylcellulose, hydroxypropyl methylcellulose, sodium carboxymethylcellulose and polyvinylpyrrolidone; and, if desired, disintegrators, e.g., starches, crosslinked polyvinylpyrrolidone, alginic acid or salts thereof. Additional suitable excipients are flow conditioners and lubricants, e.g., silicic acid, talc, stearic acid and salts thereof, such as magnesium or calcium stearate, polyethylene glycol, and derivatives thereof.

The active ingredient, i.e., a staurosporine derivative, of the present invention completely or substantially completely inhibits ocular neovascularization, especially retinal neovascularization and chorodial neovascularization. In addition, a preventive efficacy is observed when a compound of the present ivention is administered to an individual. In each case, the effect on the pathologic blood vessels is dramatic and profound with complete or near-complete inhibition, but there is no identifiable toxic effect on mature retinal vessels.

The present invention is further illustrated with the following examples. However, these are not to be construed as limiting the invention thereto.

Example 1

Ischemic retinopathy is produced in C57/BL6J mice by a method described by Smith et al., Oxygen-induced Retinopathy in the Mouse, Invest. Ophthalmol. Vis. Sci. 35, 101-111 (1994). Seven-day-old mice and their mothers are placed in an airtight incubator and

exposed to an atmosphere of 75 ± 3% oxygen for 5 days. Incubator temperature is maintained at 23 ± 2°C, and oxygen is measured every 8 hours with an oxygen analyzer. After 5 days, the mice are removed from the incubator, placed in room air, and subjected to a drug treatment. N-benzoyl-staurosporine (NBS) is dissolved in dimethyl sulfoxide (DMSO) and diluted to final concentrations with water; the maximum concentration of DMSO is 1%. Vehicle (1% DMSO) or vehicle containing various concentrations of the drug (volume = 10 µl per gram body weight) is placed in the stomach by gavage. Different mice are given 60, 300 or 600 mg of NBS per kg of body weight. As a control, a group of mice are given the vehicle without NBS.

After 5 days of treatment, the mice are sacrificed, eyes are rapidly removed and frozen in optimum cutting temperature embedding compound (OCT; Miles Diagnostics, Elkhart, IN) or fixed in 10% phosphate-buffered formalin and embedded in paraffin. Adult C57BL6J mice are also treated by gavage with the drug or vehicle and after 5 days, they are sacrificed and their eyes are processed for frozen or paraffin sections.

Frozen sections (10 µm) of the eyes from drug-treated and control mice are histochemically stained with biotinylated griffonia simplicifolia lectin B4 (Vector Laboratories, Burlingame, CA) which selectively binds to endothelial cells. Slides are incubated in methanol/H_2O_2 for 10 minutes at 4°C, washed with 0.05 M Tris-buffered saline, pH 7.6 (TBS), and incubated for 30 minutes in 10% normal porcine serum. Slides are incubated 2 hours at room temperature with biotinylated lectin and after rinsing with 0.05M TBS, they are incubated with avidin coupled to peroxidase (Vector Laboratories) for 45 minutes at room temperature. After being washed for 10 minutes with 0.05 M TBS, slides are incubated with diaminobenzidine to give a brown reaction product. Some slides are counterstained with hematoxyln and all were mounted with Cytoseal.

To perform quantitative assessments, 10 µm serial sections are cut through half of each eye and sections roughly 50-60 µm apart are stained with lectin, providing 13 sections per eye for analysis. Lectin-stained sections are examined with an Axioskop microscope (Zeiss, Thornwood, NY) and images are digitized using a 3 CCD color video camera (IK-TU40A, Toshiba, Tokyo, Japan) and a frame grabber. Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) is used to delineate lectin-stained cells on the surface of the retina and their area is measured. The mean of the 13 measurements from each eye is used as a single experimental value.

The mice with ischemic retinopathy treated with the vehicle without NBS show a marked increase in the area of endothelial cell staining throughout the retina with large

clumps of cells on the retinal surface when compared to nonischemic mice, which show normal vessels in the superficial and deep capillary beds with a few connecting vessels. Ischemic mice that are given 600 mg/kg of NBS once a day for 5 days have a dramatic decrease in endothelial cell staining on the surface and within the retina when compared to the vehicle-treated mice. In fact, the endothelial cell staining within the retina of the NBS-treated ischemic mice is less than that of the nonischemic mice. High magnification shows that there are no identifiable endothelial cells on the surface of the retina, indicating that there is complete inhibition of neovascularization. There is also a striking absence of endothelial cell staining in the inner nuclear layer and outer plexiform layer where the deep capillary beds are normally located.

The mice with ischemic retinopathy which are given 300 mg/kg or 60 mg/kg of NBS twice a day by gavage show some clumps of neovascularization on the surface of the retina that is less than clumps in the retina of the vehicle-treated control mice. The retinas of NBS-treated mice also show some decrease in endothelial staining within the retina. The result of the image analysis demonstrated that the endothelial cell staining on and in the retinas of mice treated with 600 or 60 mg/kg once a day, was significantly less than that in vehicle-treated mice and showed a dose-dependent effect when compared to the mice that were treated twice a day. The results clearly demonstrate that the staurosporine derivative inhibits retinal neovascularization.

Example 2

Adult C57BL6J mice are given the vehicle or 600 mg/kg of NBS by gavage once a day and after 5 days, they are sacrificed and their eyes are processed as in Example 1.

Image analysis shows that there is no difference in the total area of endothelial staining in the retina or the appearance of retinal vessels in the NBS-treated mice compared to vehicle-treated mice. The analysis also shows no difference in the amount of retinal endothelial cell staining between the NBS- and vehicle-treated mice. This demonstrates that the staurosporine derivative is not toxic to endothelial cells of mature vessels.

Example 3

Litters of newborn C57/BL6J mice (neonatal mice) are divided into treatment and control groups which received daily subcutaneous injections of 100 mg/kg of the drug or vehicle, respectively. At 7 or 10 days of age, the mice are anesthetized with ether, and perfused with 1 ml of phosphate-buffered saline containing 50 mg/ml of fluorescein-labeled

dextran ($2x10^6$ average mw, Sigma, St. Louis, MO) as described by Tobe et al., Evolution of Neovascularization in Mice with Overexpression of VEGF in Photoreceptors, Invest. Ophthalmol. Vis. Sci. 39, 180-188 (1998). The eyes are removed and fixed for 1 hour in 10% phosphate-buffered formalin. The cornea and lens are removed and the entire retina is carefully dissected from the eyecup. Radially cuts are made from the edge of the retina to the equator in all 4 quadrants, and the retina is flat-mounted in Aquamount with photoreceptors facing upward. The flat mounts are examined by fluorescence microscopy, and the images are digitized using a 3 CCD color video camera and a frame grabber. Image-Pro™ Plus is used to measure the distance from the center of the optic nerve to the leading front of developing retinal vessels in each quadrant and the mean is used as a single experimental value.

At 7 days of age, retinal vessels in the vehicle-treated mice almost reach the peripheral edge of retina, but in the NBS-treated mice, retinal vessels only extend slightly more than halfway to the periphery. At 10 days of age, the superficial capillary bed is complete and extends all the way to the peripheral edge of the retina, and the deep capillary bed is partially developed. But in the NBS-treated mice, the superficial capillary bed has not yet reached the edge of the retina. The distance from the optic nerve to the vascular front is calculated by image analysis and the differences between the treated and control mice at 7 and 10 days of age is statistically significant. This indicates that the staurosporine derivative inhibits retinal vascular development.

Example 4

C57BL/6J mice are treated in accordance with the Association for Research in Vision and Ophthalmology resolution for the treatment of animals. Choroidal neovascularization is generated by modification of a previously described technique, Tobe et al. Targeted disruption of the *FGF2* gene does not prevent choroidal neovascularization in a murine model, Amer. J Path, in press. Briefly, 4 to 5 week old male C57BL/6J mice are anesthetized with ketamine hydrochloride (100 mg/kg body weight) and the pupils are dilated with 1% tropicamide. Three burns of krypton laser photocoagulation (100 µm spot size, 0.1 seconds duration, 150 mW) are delivered to each retina using the slit lamp delivery system of a Coherent Model 920 Photocoagulator and a hand held cover slide as a contact lens. Burns are performed in the 9, 12, and 3 o'clock positions of the posterior pole of the retina. Production of a bubble at the time of laser, which indicates rupture of Bruch's membrane, is an important factor in obtaining CNV, so only mice in which a bubble is produced for all three burns are included. Ten mice are

randomly assigned to treatment with vehicle alone, and ten mice are assigned to receive vehicle containing 400mg/kg/day of NBS given orally by gavage. After 14 days, the mice are killed with an overdose of pentobarbital sodium, and their eyes are rapidly removed and frozen in optimal cutting temperature embedding compound (OCT). Frozen serial sections (10 μm) are cut through the entire extent of each burn and histochemically stained with biotinylated griffonia simplicifolia lectin B4 (Vector Laboratories, Burlingame, CA), which selectively binds to endothelial cells. Slides are incubated in methanol/H_2O_2 for 30 minutes at 4°C, washed with 0.05 M Tris-buffered saline, pH 7.4 (TBS), and incubated for 30 minutes in 10% normal swine serum. Slides are rinsed with 0.05M TBS and incubated 2 hours at 37°C with biotinylated lectin. After being rinsed with 0.05M TBS, slides are incubated with Streptavidin-phosphatase (Kirkegaard and Perry Laboratories, Cabin John, MD) for 30 minutes at room temperature. After a 10 minute incubation in 0.05 M Tris buffer, pH 7.6, slides are developed in Histomark Red (Kirkegaard and Perry) to give a red reaction product, and mounted with Cytoseal (Stephens Scientific, Riverdale, NJ). Some slides are counterstained with Contrast Blue (Kirkegaard and Perry).

To perform quantitative assessments, lectin-stained sections are examined with an Axioskop microscope (Zeiss, Thornwood, NY) and images are digitized using a 3 CCD color video camera (IK-TU40A, Toshiba, Tokyo, Japan) and a frame grabber. Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) is used to delineate and measure the area of lectin-stained blood vessels in the subretinal space. For each lesion, area measurements are made for all sections on which some of the lesion appeared and added together to give the integrated area measurement. Values are averaged to give one experimental value per mouse. A 2-sample t-test for unequal variances is performed to compare the log mean integrated area between treatment and control mice.

Two weeks after laser, all lesions in both groups of mice show a discontinuity in Bruch's membrane with roughly equivalent damage to the overlying retina. All mice treated with vehicle alone show large areas of choroidal neovascularization at the site of each laser-induced rupture of Bruch's membrane. There is proliferation of retinal pigmented epithelial cells along the margin of the new vessels. Retinal blood vessels stained with lectin are seen in the overlying retina. In contrast, all mice given 400 mg/kg/day of NBS have very little if any choroidal neovascularization at the site of each laser-induced rupture of Bruch's membrane. In most instances, there is no identifiable lectin-stained neovascular tissue throughout the entire burn, but some burns contained regions in which there are thin discs of lectin-stained tissue. There is mild proliferation of RPE cells. Despite the marked

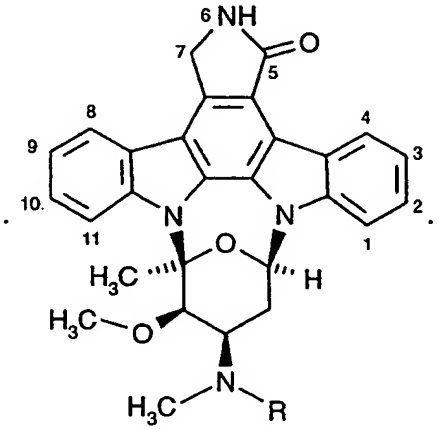
decrease in choroidal neovascularization in the eyes of treated mice, the overlying retinal vessels appear normal. This is best seen in sections with no counterstain.

Quantitation of the integrated area of lectin staining per lesion shows a dramatic decrease in the mice treated with NBS (0.0090182 ± 0.0017540 mm^2) compared to lesions in mice treated with vehicle alone (0.0695621 ± 0.0073960 mm^2). This difference is highly statistically significant ($p=0.004$). These results clearly demonstrate that the staurosporine derivative dramatically inhibits chorodial neovascularization.

The above examples illustrate the efficacy of the active ingredient. The staurosporine derivatives of the present invention are highly effective in completely or substantially completely inhibiting retinal and chorodial neovascularization, and the active ingredients can be administered to a patient by a drug treatment mode which is conventional and/or practical.

Claims

1. Use of a compound of formula (I) or a salt thereof,



(I),

wherein R represents a hydrocarbyl radical R^o or an acyl radical Ac,
in the preparation of a medicament for the treatment and/or prevention of ocular neovascular diseases.

2. The use of claim 1 wherein said hydrocarbyl radical is an acyclic, carbocyclic, carbocyclic-acyclic, heterocyclic or heterocyclic-acyclic hydrocarbyl radical.

3. The use of claim 2 wherein said acyclic hydrocarbyl radical is a radical of a C_1-C_{20}-alkyl radical, C_2-C_{20} hydroxyalkyl radical of which the hydroxy group is in any position other than the 1-position, cyano-[C_1-C_{20}]-alkyl radical, carboxy- [C_1-C_{20}]-alkyl radical of which the carboxy group, or C_3-C_{20}-alkenyl radical of which the free valency is not at the same carbon atom as the double bond.

4. The use of claim 2 wherein said carbocyclic hydrocarbyl radical is a radical of mono-, bi- or polycyclic cycloalkyl; cycloalkenyl; cycloalkandienyl; and aryl.

5. The use of claim 2 wherein said carbocyclic-acyclic radicals is an acyclic radical that carry one or more of carbocyclic radicals, and said heterocyclic radical and heterocyclic-acyclic radical are monocyclic, bicyclic, polycyclic, aza-, thia-, oxa-, thaza-, oxaza-, diaza-, triaza-, and tetraza-cyclic radicals of aromatic character.

6. The use of claim 1 wherein said acyl radical is an optionally functionally modified carboxylic acid, organic sulfonic acid, or optionally esterified phosphoric acid.

7. The use of claim 6 wherein said acyl radical has the formula Z-C(=W)-, wherein W is oxygen, sulfur, or imino and Z is hydrogen, C_1-C_7 alkyl, amino, phenyl, pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl.

8. The use of claim 6 wherein said acyl radical has the formula R_b^O-CO-, wherein R_b^O is hydrogen, benzoyl, or a C_1-C_{19} alkyl radical.

9. The use of claim 6 wherein said acyl radical has the formula R^O-O-CO-, wherein R^O is an acyclic, carbocyclic, carbocyclic-acyclic, heterocyclic or heterocyclic-acyclic hydrocarbyl radical.

10. The use of claim 6 wherein said acyl radial has the formula

$$R_1 - N(R_2) - C(=W) -$$

wherein R_1 and R_2 are independently selected from hydrogen and unsubstituted acyclic C_1-C_7 hydrocarbyl.

11. The use of claim 6 wherein said acyl radial has the formula R^O-SO_2- wherein R^O is a hydrocarbyl radical.

12. The use of claim 6 wherein said acyl radical has the formula

$$R_1O - P(=O)(OR_2) -$$

in which R_1 and R_2 are independently selected from hydrogen, unsubstituted acyclic C_1-C_7 hydrocarbyl.

13. The use of claim 1 wherein said active ingredient is selected from N-(3-carboxy-propionyl)-staurosporine, N-benzoyl-staurosporine, N-trifluoracetyl-staurosporine, N-methylaminothiocarbonyl-staurosporine, N-phenylcarbamoyl-staurosporine, N-(3-nitro-

benzoyl)-staurosporine, N-(3-fluorobenzoyl)-staurosporine, N-tert-butoxycarbonyl-staurosporine, N-(4-carboxybenzoyl)-staurosporine, N-(3,5-dinitrobenzoyl)-staurosporine, N-alanyl-staurosporine, N-ethyl-staurosporine, N-carboxymethyl-staurosporine, N-[(tert-butoxycarbonylamino)-acetyl]-staurosporine, N-(2-aminoacetyl)-staurosporine, and salts thereof.

14. The use of claim 1 wherein said active ingredient is N-benzoyl-staurosporine or a salt thereof.

15. The use of claim 1 wherein said treatment and/or prevention is the treatment and/or prevention of human ocular neovascular diseases.

16. The use of claim 1 wherein said treatment and/or prevention is the treatment and/or prevention of human retinal neovascular diseases.

17. The use of claim 1 wherein said treatment and/or prevention is the treatment and/or prevention of human chorodial neovascular diseases.

18. The use of claim 1 wherein said treatment and/or prevention is the treatment and/or prevention of mammalian ocular neovascular diseases.

19. A pharmaceutical composition for the treatment or prevention of ocular neovascular diseases, which composition comprises a therapeutically effective amount of a compound of formula (I) or a salt thereof in accordance with claim 1.

20. The pharmaceutical composition of claim 19 wherein said compound is N-benzoyl-staurosporine.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 99/08987

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/553 A61P27/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MEYER T ET AL: "A derivative of staurosporine (CGP 41 251) shows selectivity for protein kinase C inhibition and in vitro anti-proliferative as well as in vivo anti-tumor activity." INTERNATIONAL JOURNAL OF CANCER, (1989 MAY 15) 43 (5) 851-6. JOURNAL CODE: GQU. ISSN: 0020-7136., XP002102539 United States	1-15, 18-20
Y	the whole document	16,17
	-/—	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 April 2000	10/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hoff, P

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	MURPHY T L ET AL: "Migration of retinal pigment epithelium cells in vitro is regulated by protein kinase C." EXPERIMENTAL EYE RESEARCH, (1995 JUN) 60 (6) 683-95. JOURNAL CODE: EPL. ISSN: 0014-4835., XP002102540 ENGLAND: United Kingdom the whole document	16,17
X	US 5 093 330 A (CARAVATTI GIORGIO ET AL) 3 March 1992 (1992-03-03) cited in the application the whole document	19,20
Y	WOOD, J. (1) ET AL: "CGP 41251, an inhibitor of VEGF receptor tyrosine kinases." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, (1997) VOL. 38, NO. 0, PP. 266. MEETING INFO.: EIGHTY-EIGHTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH SAN DIEGO, CALIFORNIA, USA APRIL 12-16, 1997 ISSN:, XP002102541 the whole document	1-18
Y	TOBE T ET AL: "Evolution of neovascularization in mice with overexpression of vascular endothelial growth factor in photoreceptors." INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, (1998 JAN) 39 (1) 180-8. JOURNAL CODE: GWI. ISSN: 0146-0404., XP002102542 United States cited in the application the whole document	1-18
Y	WO 97 34920 A (SUGEN INC) 25 September 1997 (1997-09-25) abstract page 2, line 15 -page 10, line 27 page 23, line 12 -page 24, line 4; claims 1,16,19,20	1-18
Y	WO 97 40831 A (WAYS DOUGLAS KIRK ;KING GEORGE L (US); VIGNATI LOUIS (US); AIELLO) 6 November 1997 (1997-11-06) the whole document	1-18

-/—

1

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 99/08987

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GESCHER A: "Analogs of staurosporine: potential anticancer drugs?." GENERAL PHARMACOLOGY, (1998 NOV) 31 (5) 721-8. REF: 65 JOURNAL CODE: FLK. ISSN: 0306-3623., XP002102543 ENGLAND: United Kingdom the whole document	19,20

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter ...nal Application No
PCT/EP 99/08987

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 5093330	A	03-03-1992	AT	134375	T	15-03-1996
			AU	617324	B	28-11-1991
			AU	1757188	A	15-12-1988
			CA	1337763	A	19-12-1995
			DE	3855015	D	28-03-1996
			DK	324888	A	16-12-1988
			EP	0296110	A	21-12-1988
			ES	2083956	T	01-05-1996
			FI	882808	A,B,	16-12-1988
			GR	3019064	T	31-05-1996
			HK	1003788	A	06-11-1998
			HR	940452	A	30-04-1997
			IE	70523	B	11-12-1996
			JP	1034989	A	06-02-1989
			JP	2708047	B	04-02-1998
			KR	9701529	B	11-02-1997
			NO	882613	A,B,	16-12-1988
			NZ	225018	A	26-09-1990
			PT	87719	A,B	01-07-1988
			SI	8811154	A,B	31-10-1996
			YU	115488	A	31-12-1989
			DD	281808	A	22-08-1990
			HU	201329	B	28-10-1990
			MX	11907	A	31-01-1994
			ZA	8804238	A	22-02-1989
WO 9734920	A	25-09-1997	AU	2066797	A	10-10-1997
WO 9740831	A	06-11-1997	AU	2935597	A	19-11-1997
			AU	2936197	A	19-11-1997
			AU	3000297	A	19-11-1997
			CN	1233177	A	27-10-1999
			CZ	9803443	A	14-07-1999
			CZ	9803499	A	17-11-1999
			CZ	9803500	A	17-11-1999
			EP	0915698	A	19-05-1999
			EP	0918519	A	02-06-1999
			EP	0914135	A	12-05-1999
			HU	9902315	A	29-11-1999
			NO	985065	A	28-12-1998
			NO	985066	A	21-12-1998
			NO	985067	A	22-12-1998
			PL	329851	A	12-04-1999
			PL	330463	A	24-05-1999
			PL	330862	A	07-06-1999
			WO	9740830	A	06-11-1997
			WO	9740842	A	06-11-1997